Dietary acid load and bone turnover during long-duration spaceflight and bed rest.

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Short Running Head: Dietary acid load and bone turnover in spaceflight

Abbreviations: APro:K, animal protein:potassium; ARED, Advanced Resistive Exercise Device; BMC, bone mineral content; BMD, bone mineral density; BSAP, bone-specific alkaline phosphatase; BW, body weight; DXA, dual-energy x-ray absorptiometry; FD, flight day; HP, helical peptide; ISS, International Space Station; ITS-CRC, Institute for Translational Sciences-Clinical Research Center; JSC, Johnson Space Center; NASA, National Aeronautics and Space Administration; NCC, Nutrition Coordinating Center; NEAP, net endogenous acid production; NTX, N-telopeptide; pQCT, peripheral quantitative computed tomography; WAFAL, Water & Foods Analytical Laboratory.

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Abstract [max 300 wd; complete sentences required; now 312 wd]

Background: Bed rest studies document a lower dietary acid load is associated with lower bone resorption.

Objective: We tested the effect of dietary acid load on bone metabolism during spaceflight.

Design: Controlled 4-d diets with a High or Low ratio of animal protein to potassium (APro:K) were given to 17 astronauts before and during spaceflight. Each astronaut had 1 High and 1 Low diet session before flight and 2 High and 2 Low sessions during flight. In a fifth 4-d session around flight day 30 (FD30), crewmembers were to consume their typical in-flight intake. At the end of each session, blood and urine samples were collected. Calcium, total protein, energy, and sodium were maintained in each crewmember's preflight and in-flight controlled diets.

Results: N-telopeptide (NTX) and urinary calcium were higher during flight. Bone-specific alkaline phosphatase (BSAP) was higher toward the end of flight. The High and Low diets did not have an effect on NTX, BSAP, or urine calcium. Dietary sulfur and age were significantly associated with changes in NTX. Dietary sodium and flight day were significantly associated with urinary calcium during flight. The net endogenous acid production (NEAP) estimated from the typical dietary intake at FD30 was associated with loss of bone mineral content in the lumbar spine after the mission. Resistive exercise during flight is likely a confounding factor. The results were compared to data from a 70-d bed rest study, in which control (but not exercising) subjects' APro:K was associated with higher NTX during bed rest.

Conclusions: Long-term lowering of NEAP by increasing vegetable and fruit intake may protect against changes in loss of bone mineral content during spaceflight when adequate calcium is consumed, particularly if resistive exercise is not being performed, but further investigation is needed.

Keywords: animal protein, bone mineral content, bone resorption, bone-specific alkaline phosphatase, dietary acid, dietary sulfur, N-telopeptide, net endogenous acid production, resistive exercise, weightlessness

Introduction

Though the mechanism of bone mineral loss associated with spaceflight is not completely understood, it is clearly multifactorial. With adequate nutritional support, resistive exercise can mitigate decrements in bone mineral density (BMD) during spaceflight (1, 2), and it is likely that diet can be optimized to further protect bone (3, 4). While pharmacological agents provide an alternative in the event of exercise device failure (5), avoiding these agents in otherwise healthy, relatively young individuals seems prudent. Dietary effects on bone are well established, and have virtually no risk of side effects.

Ground-based evidence supports the hypothesis that a suboptimal diet is associated with lower bone mineral status and higher osteoporosis and fracture risk in healthy subjects (6). Mediterranean diets rich in fruits and vegetables are generally beneficial to bone (7-9), possibly because low endogenous acid production is associated with fruit and vegetable intake. Endogenous acids include sulfuric acid produced from sulfur-containing proteins and amino acids. Generally, foods containing animal protein are higher in sulfur-containing amino acids than plant protein sources, and plants have a higher content of alkaline precursors. Phosphoric acid is another nonvolatile acid that can either be ingested in the diet or produced endogenously. Anions, including conjugate bases of organic acids, make up the majority of dietary base precursors that the body metabolizes to bicarbonate. Potassium is the predominant intracellular inorganic cation that balances the charge of organic anions; therefore, dietary potassium intake can be used to estimate the content of base precursors in the diet. Frassetto and colleagues developed a model for estimating net endogenous acid production (NEAP) on the basis of the acid and base precursors in the diet (10). According to this model, renal net acid excretion can be predicted from 2 dietary components: total protein and potassium.

In bed rest, a common analog of spaceflight effects on bone, we showed that a higher ratio of dietary animal protein to potassium (Apro:K) was associated with more excretion of both calcium and collagen crosslinks, but had no association with markers of bone formation (11, 12). While this observation was clear after 2-3 wk of bed rest, it was not as profound before bed rest when the subjects were ambulatory. This suggests that the impact of diet on bone is more pronounced in non-exercising individuals, specifically those whose bone is in a resorptive state, as is the case during bed rest. In a separate study in which bed rest subjects were supplemented with essential amino acids including methionine (a sulfur-containing amino acid), urine pH was lower and markers of bone resorption were higher in subjects receiving the supplement, while bone formation was not affected (13).

On the basis of these findings, we proposed that the ratio APro:K in the diet would be associated with bone metabolism during spaceflight. We report here results from a controlled diet study in which we investigated acute effects of controlled diets on biochemical markers of bone metabolism. For comparison, similar data from a 70-d ground-based bed rest study evaluating exercise effects on bone and other systems are also presented.

Subjects and Methods

Seventeen International Space Station (ISS) astronauts (13 male and 4 female, 47 ± 6 y at the time of launch, BMI 24.6 ± 3.0; mission durations of 160 ± 20 d, mean ± SD) participated in the study. The protocol was reviewed and approved by the National Aeronautics and Space Administration (NASA) Johnson Space Center (JSC) Institutional Review Board, the Japanese Aerospace Exploration Agency, and the European Space Agency Medical Boards. Written informed consent was obtained from all crewmembers before their participation. While we have published other bone and related data from astronauts on ISS missions, none of the data reported here have been previously published, and none of the data from the astronauts included here were included in those other publications.

Dietary treatments, recording, and analysis

The experimental goal was to test 2 diets: one (“High”) with a “high” ratio of animal protein:potassium (1.0-1.3 g animal protein/mEq potassium), and one (“Low”) with a “low” ratio (0.3-0.6 g/mEq). The ranges were selected to represent the high end and low end of the linear relationship between dietary APro:K and NTX from a bed rest study (11). Four-day menus for each diet were developed from available space foods, and foods for each crewmember were developed from the same lot for both the preflight and in-flight controlled-diet sessions. The High and Low 4-d menus for each crewmember were designed to provide similar (within 5% of each other) intakes of total energy (based on WHO calculations for that crewmember (14)), total protein, sodium, and calcium intakes within a crewmember. A research dietitian met with each crewmember in advance to plan acceptable High and Low menus given the available space foods. Identical menus were provided in a semi-randomized crossover fashion, such that each crewmember had 1 High and 1 Low diet session before flight and 2 High and 2 Low sessions during flight. The randomization was stunted to yield (roughly) equal numbers of High and Low diet sessions at each of the designated time points during flight. The preflight sessions occurred at about 6 mo and at 3 mo before the mission, and the in-flight sessions occurred around flight day (FD) 15, FD60, FD120, and FD180. All crewmembers completed at least 3 controlled diet sessions, but not all crewmembers completed all 4 controlled diet intake sessions during flight due to the shorter durations of some missions. Note that, although 7, 8, or 9 subjects participated in each of the High or Low sessions, because of randomization participants were not the same individuals from one session to the next.

In addition to the controlled dietary intake sessions during flight, there was a 4-d session at FD30 when crewmembers had no dietary restrictions but during those 4 d were asked to consume and record their typical intake. This session was intended to provide an understanding of the crewmembers' typical intake during the mission. Crewmembers completed similar 4-d monitored intake sessions after flight (return plus (R+) 30, 180, 365) (data not reported here). A fasting blood sample and a 24-h urine collection were also obtained about 10-45 d before flight with no diet monitoring (and no diet restrictions).

Dietary intake data were collected and analyzed using Nutrition Data System for Research software versions 2007, 2010, 2012, and 2014 developed by the Nutrition Coordinating Center (NCC), University of Minnesota, Minneapolis, MN. Space foods are analyzed in the Water & Food Analytical Laboratory (WAFAL) at NASA JSC for macronutrient and mineral content including moisture, fat (total, saturated, and unsaturated), fiber, protein, carbohydrate, calories, calcium, magnesium, sodium, potassium, phosphorus, copper, iron, manganese, zinc, chloride, iodine, fatty acid profile, and cholesterol. Recipes and nutrients analyzed by the WAFAL lab for each food item were submitted to the NCC and the content of other nutrients was imputed according to the analyzed data and recipes. Dietary sulfate was determined using an equation from Remer and colleagues (15). Diets were designed for each individual crewmember according to their estimated energy requirements (14) and study constraints. In some cases, however, a crewmember simply could not consume the prescribed amount of food, and in other cases crewmembers reported being hungry at the end of the day when the allotted food items were depleted. In the former cases, attempts were made to adjust menus for subsequent sessions to maintain study constraints at lower energy intakes. In the latter cases, non-protein-containing food items were provided to increase calories. Actual intakes are reported in the results.

All crewmembers were provided with vitamin D supplements (800 IU/d) during flight. They were asked to refrain from taking any other supplements during the 4-d controlled dietary intake sessions.

NEAP was calculated from dietary components using an equation from Remer and Manz (15), which incorporates the potential renal acid load of the diet and the diet-independent organic acid excretion estimated from body surface area.

Biological Sample Collections and Biochemical Analyses

At the end of each 4-d session, 24-h urine samples and fasting blood samples were collected, with the closing urine void collected at around the same time as the fasting blood draw. We have previously reported the details of techniques and equipment used for in-flight biological sample collections and processing (1, 16).

Blood samples were analyzed for bone biochemical markers using standard techniques, as described previously (1, 2). Urine samples were analyzed for collagen crosslinks, including NTX, C-telopeptide, and helical peptide (HP), using commercially available kits (Osteomark, Ostex International, Seattle, WA; Osteometer BioTech, Herley, Denmark; and Quidel, San Diego, CA, respectively). Urinary sulfate was measured as previously described (17). Urinary pH was measured in 24-h urine collections using a standard pH meter. Urine pH was determined during flight using a paper strip technique, but we found that the lighting on board the ISS coupled with the color gradations on the strip did not allow clear resolution of pH.

Exercise

All crewmembers regularly performed resistive exercise on the ISS Advanced Resistive Exercise Device (ARED) (2, 18). The types of exercise performed on the ARED include squats, heel raises, deadlift, shoulder press, abdominals, and bent-over row. All exercises performed on the ARED were included in the reported totals. The amount of exercise was determined by multiplying the amount of weight used by the number of repetitions and sets for each exercise performed on the device. The daily total pounds lifted in the 3 wk before the blood and urine collection associated with each dietary session was then calculated. If less than 3 wk of data were available (i.e., for the FD15 session), then only the available data were used.

Bone Densitometry

BMC and BMD were assessed about 3 mo before and within 1 mo after each flight by dual-energy x-ray absorptiometry (DXA) with a fan beam densitometer (Hologic Discovery; Hologic, Inc., Waltham, MA, USA) as described previously (2).

Environmental Data

ISS cabin carbon dioxide (CO_2) was determined by the ISS Cabin Gas Analyzer and by the Major Constituent Analyzer. The average of the 2 measurements in the 24-h day of each blood draw was used in the analyses.

Bed Rest

Comparison data are reported here from a bed rest study evaluating exercise as a countermeasure to physiological effects of simulated microgravity (19). These data are from one of a series of bed rest studies conducted at the University of Texas Medical Branch (UTMB) in Galveston, TX (20). Protocols were reviewed by the NASA JSC and UTMB institutional review boards, and written informed consent was obtained before participation.

While we and others have reported data from this (19) and similar (21, 22) bed rest studies, to our knowledge, none of the data reported here have been published previously (and certainly no data pertaining to dietary APro:K effects on bone).

Subjects (n=11, 10 M, 1 F; ages 38 ± 7; BMI 25.2 ± 2.2) participated in a 70-d 6° head-down-tilt bed rest study. Five subjects completed daily supine exercise, which started on the first day of bed rest, and 6 subjects served as non-exercising controls. The exercise consisted of aerobic and resistance exercise (eg, squat, heel raise, leg press, cycling, and treadmill), designed to be similar to the exercise profiles being tested on the ISS.

Subjects were housed in the Institute for Translational Sciences-Clinical Research Center (ITS-CRC) at UTMB (Galveston, TX) for at least 13 d before the start of bed rest until 6 d after reambulation following the 70-d bed rest phase of the study. Food was weighed and dietary intake was recorded daily by metabolic kitchen staff, as described for similar bed rest studies in the ITS-CRC (23). These diets were controlled, but not with respect to the APro:K ratio. For this report, the dietary intake data were analyzed for the 7 d before the urine collection and an average APro:K was determined. Urine was collected in 24-h pools 4 times before bed rest (collected 10, 9, 3, and 2 d before bed rest) and monthly during bed rest. For this report, the pre-bed rest NTX average represents the average NTX of the 4 days before bed rest, and the bed rest NTX is the average of the last 2 days of bed rest (bed rest days 69 and 70).

Statistical Analyses

To test for differences in demographic, in-flight exercise, environmental, and reported dietary intake measures between astronauts assigned to High and Low APro:K diets at FD15, FD60, FD120, and FD180, we used 2-sided t-tests and Fisher's exact tests for continuous and discrete data, respectively. Additionally, we used 2-sided, paired t-tests to investigate differences between astronauts' mean in-flight exercise, environmental, and dietary intake measures while they were on the monitored diet session at FD30 and the same measures while they were on High or Low APro:K diets at FD15, FD60, FD120, and FD180. To control the familywise error rate, we adjusted the α-level of the tests using a false discovery rate adjustment (24): we restricted the false discovery rate to 0.05, which means that we would expect 5% of the rejected null hypotheses to be false (incorrect rejection).

Two mixed-effects linear regression models were fit using the **lme4** package (25) in R (26) to assess differences in the changes in urinary NTX, BSAP, and daily urinary calcium excretion from baseline across assigned diets (High APro:K, Low APro:K, monitored) for measures collected before and during flight (first model), and for in-flight measures only (second model). All dependent variables (urinary NTX, BSAP, urinary calcium) were normalized by subtracting astronauts' respective baseline measures collected 10 d before flight, when they were not on a controlled (or monitored) diet. We incorporated a random intercept term in each model, which accommodated random heterogeneity in astronauts' changes in NTX, BSAP, and urinary calcium from baseline that persisted throughout the study. Diet was treated as a 3-level categorical covariate and modeled using indicator variables, with the monitored diet session serving as the reference category. Each model controlled for fixed effects of age, body mass (kg), sex (male as reference), energy (kcal/d), total protein (g), dietary sulfur (mEq/d), dietary potassium (mg), dietary calcium (mg), dietary sodium (mg), NEAP, and flight day. For the first (preflight and in-flight) model, we incorporated a main effect for in-flight status (using preflight data as reference) and an interaction between in-flight status and relative day from launch when significant. For the in-flight-only models, we additionally controlled for cumulative 3 wk of exercise (lbs) and average CO_2 (mmHg). *P* values for significance of the regression coefficient were obtained using the Kenward-Roger approximation with R package **pbkrtest** (27). Significance was determined at the 0.05 α-level.

FD30/DXA analysis

We assessed whether changes in BMC and BMD after spaceflight were associated with dietary protein using simple linear regression models. Thereafter, we fit multiple regression models to investigate whether dietary sulfur confounded these relationships. Additionally, we took a similar approach to assess whether changes in total lumbar spine BMC were associated with NEAP at FD30, controlling for age and sex. Significance was determined at the 0.05 α-level.

Bed Rest Analyses

Pearson correlation coefficients were determined for the control and exercise groups for the 7-d mean APro:K (7 d before urine collections) and the average pre-bed rest NTX as well as the average NTX from bed rest days 69 and 70.

Results

All crewmembers completed all planned sessions, although 3 had truncated mission durations and as a result could not complete an FD180 session. Demographic, in-flight exercise and CO_2, and summary dietary intake data are shown in **Table 1**. An extended set of intake data are provided in **Supplemental Table 1**. It is important to recall that crewmembers did not follow the same pattern of diets, and thus the subjects in the High or Low groups were not the same individuals from one session to the next. This was accounted for in the analysis.

Energy intake was generally maintained during High and Low APro:K diet sessions, but small adjustments were made when crewmembers felt they could not consume the food that met estimated energy requirements or when crewmembers requested additional food because they were hungry. Energy intake during the controlled sessions was higher than in the FD30 monitored intake session (Table 1). As designed, dietary total protein, calcium, and sodium were maintained during crewmembers' controlled dietary intake sessions. NEAP was different between the 2 diets, significantly higher with High than with Low menus, as expected (Table 1).

ISS cabin CO_2 concentration was generally constant between sessions (Table 1). With an average close to 3 mm Hg on the ISS, CO_2 concentration was much higher than terrestrial CO_2 at standard pressure (0.3 mm Hg).

We evaluated 3 key bone markers: urinary NTX and calcium, and serum BSAP. Summaries of the measures collected are presented in **Figure 1** and **Table 2**, and the results of the linear mixed-effects model for preflight and in-flight measures as well as the model with only in-flight measures are shown in **Tables 3-5**.

As expected, NTX increased during flight (raw and change data are presented in **Table2**, statistical models are presented in **Table 3**). In the preflight and in-flight model (**Table 3**), the High ($P = 0.06$) and Low ($P = 0.39$) diets were not significantly associated with changes in NTX compared to the monitored diet on FD30. However, age ($P < 0.01$) and dietary sulfur ($P = 0.03$) were significantly associated with changes in NTX from baseline. Specifically, in looking at the estimate numbers in Table 3, for every 10 mEq/d increase in dietary sulfur and year increase in age, we would expect an 80 and 18 nmol/d increase in excretion of NTX, respectively, for the typical astronaut, holding all else constant. When we investigated the potential confounding effects of exercise and CO_2 exposure (looking at in-flight data only), the High Apro:K diet was associated with a decrease in NTX. However, further investigation revealed that this relationship was potentially driven by 1 individual who had a sharp increase in NTX from baseline when not on a controlled diet at FD30 (see **Supplemental Figure 1**). Refitting the model with the individual removed negated the significant difference ($P = 0.130$, Table 3). For the in-flight-only model, only age remained significantly associated with changes in NTX from baseline ($P = 0.02$, Table 3). However, the magnitudes of the effects were relatively consistent between the preflight and in-flight model and the in-flight only model. The same model was applied to urinary CTX and helical peptide, 2 additional markers of bone resorption, and the results were generally similar to what was observed for NTX (data not shown).

The data did not provide enough evidence to suggest that either the High or the Low APro:K controlled diet was significantly associated with differences between serum BSAP during flight and serum BSAP during the FD30 monitored session, in either model (**Table 4**). BSAP increased during flight (Table 2), and increased further with increased duration of flight ($P < 0.0001$ for flight day in the pre- and in-flight model). For the in-flight only model, flight day remained significant ($P < 0.001$) and CO_2 also had a small but significant ($P = 0.01$) association with BSAP (Table 4). For every 10-d increase in days of flight, we would expect a 1-U/L increase in BSAP for the typical astronaut, holding all else constant. Also, for every 1-mmHg increase in average CO_2 during flight, we would expect a 3-U/L increase in BSAP for the typical astronaut, holding all else constant.

The urinary calcium data also did not provide enough evidence to suggest that either the High or the Low APro:K controlled diet was significantly associated with differences between urinary calcium excretion during flight and urinary calcium during the FD30 monitored session (**Table 5**); however, higher dietary sodium was associated with higher urinary calcium ($P < 0.05$) in both of the mixed-effects models. Urinary calcium was significantly higher during flight than before flight ($P < 0.0001$), but there was a significant interaction between flight day and in-flight status ($P < 0.0001$). Thus, as time in flight (flight day) increased, urinary calcium increased at a slower rate (change and percentage change decreased). When looking at the in-flight only model, which controlled for CO_2 and exercise, there was still no statistically significant difference between the High or the Low Apro:K diet and a monitored diet, for urinary calcium. Dietary sodium and flight day remained significant during flight.

Dietary intake from the FD30 monitored session, used as a representation for typical in-flight dietary intake, revealed that a higher acid load (estimated by NEAP) was associated with a greater decrease in BMC of the lumbar spine (**Figure 2**, $P < 0.01$).

In the bed rest study, APro:K was not correlated with urinary NTX before bed rest, but was correlated with NTX in the control (no exercise) subjects during bed rest ($r = 0.82$, $P < 0.05$) (**Figure 3**). This effect was not observed in exercising subjects, and during bed rest those subjects showed a trend for a negative correlation ($r = -0.84$, $P = 0.07$). The bed rest dietary intake data are presented in **Supplemental Table 2**.

Discussion

The literature on the effects of protein (type and amount) on bone are mixed, with some studies demonstrating that diets providing a high protein intake are detrimental to bone (28, 29); conversely, many studies report high protein intake having a protective effect on bone (30, 31). In light of our previous bed rest data showing that the ratio of dietary APro:K was positively associated with urinary NTX excretion (11), we hypothesized that a higher APro:K diet during spaceflight would yield a greater change in urinary NTX excretion than a diet with lower APro:K, and we hypothesized that diet would have no effect before flight. Contrary to our hypothesis, we did not observe any differences in the effect on bone turnover markers when crewmembers were on a High or a Low APro:K ratio controlled diet for 4-d periods during flight. Rather than concluding that our findings support the argument that there is no effect, or a positive effect, of protein on bone, we rather maintain that these results help to clarify the discord in the literature.

The majority of the studies reporting detrimental effects of protein on bone had less than optimal calcium intake, and the studies reporting beneficial effects on bone had calcium intakes adequate to support bone formation (32-34). Jonge and colleagues (35) found that dietary acid load may be associated with negative bone outcomes only in subgroups of individuals. They found that in individuals with higher intakes of dietary fiber, a higher NEAP was more detrimental to bone; this result was hypothesized to be caused by inhibited intestinal absorption of calcium (35). In our study, dietary fiber was twice as high in the Low menus as in the High menus. We included dietary fiber in the statistical models, but it was not significant for any of the outcomes studied. It is also important to point out that crewmembers in this flight study were consuming high-protein diets overall, relative to the current RDA of 0.8 g protein/kg. The average protein intake during the controlled diet sessions was 1.5 ± 0.2 g/kg body weight (BW) (1.2 ± 0.4 g/kg BW for the FD30 session). Also, average calcium intake was 1275 ± 229 mg/d (1118 ± 397 mg/d during the FD30 session), which is more than sufficient to support bone formation. High protein intake is often argued to be detrimental to bone because it is often associated with increased urinary calcium excretion. For every 10-g increase in dietary protein, urinary calcium increases by 16 mg (36), and because excess dietary protein is catabolized to ammonium ion and sulfates from the sulfur-containing amino acids, it has been hypothesized that bone is resorbed to neutralize the dietary acid load. A key consideration is that increased dietary protein increases calcium absorption in some studies (37-39). In our study, when comparing dietary effects on NTX before and during flight, we found that a higher dietary sulfur intake was associated with an increase in urinary NTX excretion, but it was not associated with urinary calcium. Dietary sodium had a small but significant positive association with urinary calcium. Higher dietary sodium was not associated with a greater change in NTX, though, so it is possible that the calcium could come from increased intestinal absorption instead of being released from bone. We previously documented that calcium absorption decreased during spaceflight, but that was in crewmembers who did not have access to resistance exercise (40, 41). Interestingly, Thorpe and colleagues found a positive association of lumbar spine BMD with dietary protein in postmenopausal women consuming adequate calories, but the association was suppressed by a negative association with protein sulfur. Sulfur intakes were around 30 mEq/d in that study, which was about half of the sulfur intake of the crewmembers in this study. We attempted to reproduce these findings with the monitored intake data at FD30, but the total protein and sulfur were highly correlated (Pearson's $r = 0.9968$), and therefore we could not effectively tease out effects of sulfur and protein.

Exercise, or even degree of ambulation, is a major factor in these studies. Exercise is an operational countermeasure on the ISS, meaning that all crewmembers on the ISS are required to exercise – using both aerobic and resistive exercise devices before and throughout their missions (42, 43). In this study, all crewmembers had access to heavy resistance exercise, which has been shown to mitigate bone loss during flight (2, 41) and in bed rest (44). Our previous bed rest studies showed the positive relationship between APro:K and NTX, and this relationship was more pronounced in control subjects than in exercising subjects (11), and more pronounced in men (11) than women (12). The exercise in those studies was a unique protocol combining aerobic (treadmill) exercise with lower-body negative pressure. In the bed rest study reported here, diet had no effect on bone resorption during the ambulatory pre-bed rest phase. During bed rest, there was a significant association of diet and bone resorption in sedentary subjects, which was absent in exercising subjects.

Because the High and Low diets were only 4 d and not representative of the entire mission intake, we could not look at those diets with respect to overall changes in BMC. The FD30 monitored dietary intake session is the closest estimate we have to give a snapshot of the typical intake of the crewmembers during the other ~160 d of the mission when their diets were not controlled for this study. For the 4 d of that monitored session, crewmembers were asked to record all fluid and food intake with no restrictions on what they could have. The NEAP average from FD30, estimated from acid and base components of the diet, was negatively associated with the change in lumbar spine BMC. We specifically looked at lumbar spine BMC because in previous studies it was the region with the highest percentage change from preflight values for crewmembers using the ARED (2). Total BMD and BMC were not related to NEAP, and it is possible that diet effects on these whole-body measurements are swamped by the protective effect of exercise. Furthermore, Remer and colleagues (45) have suggested that the potential renal acid load of the diet may be more related to the cortical area of the bone (bone size) as assessed by peripheral quantitative computed tomography (pQCT), and not to areal BMD as is assessed by DXA. This hypothesis is based on previous pQCT findings that dietary potential renal acid load was associated with cortical area and BMC but not with volumetric cortical BMD (46). Remer et al. argue that true volumetric density, bone thickness, and bone size are integrated into a single number with DXA, and the acid load of the diet may be more closely related to bone size and bone mass than to the BMD measurement (45). We unfortunately don’t have pQCT data for the crewmembers in this study, but the relationship between dietary acid load and impact on bone is worthy of future testing.

A potential limitation/confounding factor of the present study could be that the 4-d controlled dietary sessions were simply not long enough to observe an effect on bone turnover markers. Studies have documented rapid (ie, within days) increases in bone resorption response to bed rest (47) and response to dietary changes during bed rest (48, 49), but response to dietary changes with 4-d menus has not been evaluated during spaceflight. In light of the observation that FD30 NEAP was associated with BMC loss, and assuming that intake during the FD30 session accurately represented the typical intake for the entire mission, it is possible that we simply missed this relationship with the biochemical markers after 4 d of controlled diets, and that 4 d was not long enough to observe the effect on bone biochemistry, or even that the NEAP of the diet should have been the driving factor behind the High and Low diets instead of Apro:K. Now that detailed, daily dietary intake logging has been initiated on the ISS (as opposed to weekly questionnaires), more detailed analyses regarding the effect of overall mission dietary intake on bone and other systems will be possible.

An abundance of literature exists on both sides of the protein/bone question. We have long maintained that spaceflight and flight analog research highlights the fact that neither side is “right,” and that the effect of protein on bone depends on many factors – key among them being adequacy of other dietary components (calcium, energy, etc) and ambulation (50, 51). While we had hoped this study would yield a dietary countermeasure for spaceflight-induced bone loss, and expected the microgravity environment to offer a better model of bone loss than bed rest, it seems the effects of exercise in protecting BMD during flight (2) swamp the dietary effects on bone. While this result further documents the complexity of this relationship, it does not dismiss our basic tenet that providing a long-term diet rich in fruits and vegetables will have a positive impact on bone on extended-duration missions. Such a diet would also be beneficial for other biological systems.

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Conflict of interest statement:

All authors declare that they have no potential conflicts of interest.

Authors' contributions to the manuscript:

SMS, SRZ, MH, and LCS designed research; SMS and SRZ oversaw data collection and management; BLR and HD designed menus; MK performed statistical analysis. All authors were involved in interpreting the data and preparing the manuscript. All authors read and approved the final manuscript. SMS had primary responsibility for final content.

References

1. Smith SM, Heer M, Shackelford LC, et al. Bone metabolism and renal stone risk during International Space Station missions. Bone 2015;81:712-20.
2. Smith SM, Heer MA, Shackelford LC, Sibonga JD, Ploutz-Snyder L, Zwart SR. Benefits for bone from resistance exercise and nutrition in long-duration spaceflight: evidence from biochemistry and densitometry. J Bone Miner Res 2012;27:1896-906.
3. Smith SM, Heer M, Zwart SR. Nutrition and bone health in space. In: Holick M, Nieves J, eds. Nutrition and bone health, 2nd ed. New York: Springer, 2015:687-705.
4. Orwoll ES, Adler RA, Amin S, et al. Skeletal health in long-duration astronauts: nature, assessment and management recommendations from the NASA bone summit. J Bone Miner Res 2013;28:1243-55.
5. Leblanc A, Matsumoto T, Jones J, et al. Bisphosphonates as a supplement to exercise to protect bone during long-duration spaceflight. Osteoporos Int 2013;24:2105-14.
6. Movassagh EZ, Vatanparast H. Current evidence on the association of dietary patterns and bone health: a scoping review. Adv Nutr 2017;8:1-16.
7. Byberg L, Bellavia A, Larsson SC, Orsini N, Wolk A, Michaelsson K. Mediterranean diet and hip fracture in swedish men and women. J Bone Miner Res 2016;31:2098-2105.
8. Fernandez-Real JM, Bullo M, Moreno-Navarrete JM, et al. A mediterranean diet enriched with olive oil is associated with higher serum total osteocalcin levels in elderly men at high cardiovascular risk. J Clin Endocrinol Metab 2012;97:3792-8.
9. Rivas A, Romero A, Mariscal-Arcas M, et al. Mediterranean diet and bone mineral density in two age groups of women. Int J Food Sci Nutr 2013;64:155-61.
10. Frassetto LA, Todd KM, Morris RC, Jr., Sebastian A. Estimation of net endogenous noncarbonic acid production in humans from diet potassium and protein contents. Am J Clin Nutr 1998;68:576-83.
11. Zwart SR, Hargens AR, Smith SM. The ratio of animal protein intake to potassium intake is a predictor of bone resorption in space flight analogues and in ambulatory subjects. Am J Clin Nutr 2004;80:1058-65.
12. Zwart SR, Hargens AR, Lee SM, et al. Lower body negative pressure treadmill exercise as a countermeasure for bed rest-induced bone loss in female identical twins. Bone 2007;40:529-37.
13. Zwart SR, Davis-Street JE, Paddon-Jones D, Ferrando AA, Wolfe RR, Smith SM. Amino acid supplementation alters bone metabolism during simulated weightlessness. J Appl Physiol 2005;99:134-40.
14. World Health Organization. Energy and protein requirements. Report of a joint FAO/WHO/UNU expert consultation. Geneva, Switzerland: World Health Organization, 1985.
15. Remer T, Manz F. Potential renal acid load of foods and its influence on urine pH. J Am Diet Assoc 1995;95:791-7.
16. Smith SM, Heer M, Wang Z, Huntoon CL, Zwart SR. Long-duration space flight and bed rest effects on testosterone and other steroids. J Clin Endocrinol Metab 2012;97:270-8. Erratum JCEM 97:3390. 2012.
17. Morgan JL, Heer M, Hargens AR, et al. Sex-specific responses of bone metabolism and renal stone risk during bed rest. Physiol rep 2014;2:1-12.
18. Loehr JA, Lee SM, English KL, et al. Musculoskeletal adaptations to training with the advanced resistive exercise device. Med Sci Sports Exerc 2011;43:146-56.
19. Taibbi G, Cromwell RL, Zanello SB, et al. Ophthalmological evaluation of integrated resistance and aerobic training during 70-day bed rest. Aerosp Med Hum Perform 2017;88:633-640.

20. Meck JV, Dreyer SA, Warren LE. Long-duration head-down bed rest: project overview, vital signs, and fluid balance. Aviat Space Environ Med 2009;80:A1-A8.
21. Spector ER, Smith SM, Sibonga JD. Skeletal effects of long-duration head-down bed rest. Aviat Space Environ Med 2009;80:A23-28.
22. Zwart SR, Oliver SAM, Fesperman JV, et al. Nutritional status assessment before, during, and after long-duration head-down bed rest. Aviat Space Environ Med 2009;80:A15-22.
23. Inniss AM, Rice BL, Smith SM. Dietary support of long-duration head-down bed rest. Aviat Space Environ Med 2009;80:A9-14. Erratum in Aviat Space Environ Med. 2014;85(7):768. .
24. Benjamini Y, Yekutieli D. The control of the false discovery rate in multiple testing under dependency. Ann Statistics 2001;29:1165-88.
25. Bates D, Maechler M, Bolker B, et al. Package ‘lme4’. R Foundation for Statistical Computing, Vienna 2012;12.
26. Team RC. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing, 2017.
27. Halekoh U, Højsgaard S. A Kenward-Roger approximation and parametric bootstrap methods for tests in linear mixed models – the R package pbkrtest. J Stat Softw 2014;59:1-30.
28. Feskanich D, Willett WC, Stampfer MJ, Colditz GA. Protein consumption and bone fractures in women. Am J Epidemiol 1996;143:472-9.
29. Sellmeyer DE, Stone KL, Sebastian A, Cummings SR. A high ratio of dietary animal to vegetable protein increases the rate of bone loss and the risk of fracture in postmenopausal women. Study of Osteoporotic Fractures Research Group. Am J Clin Nutr 2001;73:118-22.
30. Sukumar D, Ambia-Sobhan H, Zurfluh R, et al. Areal and volumetric bone mineral density and geometry at two levels of protein intake during caloric restriction: A randomized, controlled trial. J Bone Miner Res 2011;26:1339-48.
31. van den Hooven EH, Ambrosini GL, Huang RC, et al. Identification of a dietary pattern prospectively associated with bone mass in Australian young adults. Am J Clin Nutr 2015;102:1035-43.
32. Mangano KM, Sahni S, Kerstetter JE. Dietary protein is beneficial to bone health under conditions of adequate calcium intake: an update on clinical research. Curr Opin Clin Nutr Metab Care 2014;17:69-74.
33. Nicoll R, McLaren Howard J. The acid-ash hypothesis revisited: a reassessment of the impact of dietary acidity on bone. J Bone Miner Metab 2014;32:469-75.
34. Dawson-Hughes B. Interaction of dietary calcium and protein in bone health in humans. J Nutr 2003;133:852S-854S.
35. de Jonge EA, Koromani F, Hofman A, et al. Dietary acid load, trabecular bone integrity, and mineral density in an ageing population: the Rotterdam study. Osteoporos Int 2017;28:2357-2365.
36. Massey LK. Dietary animal and plant protein and human bone health: a whole foods approach. J Nutr 2003;133:862S-865S.
37. Cao JJ, Johnson LK, Hunt JR. A diet high in meat protein and potential renal acid load increases fractional calcium absorption and urinary calcium excretion without affecting markers of bone resorption or formation in postmenopausal women. J Nutr 2011;141:391-7.
38. Hunt JR, Johnson LK, Fariba Roughead ZK. Dietary protein and calcium interact to influence calcium retention: a controlled feeding study. Am J Clin Nutr 2009;89:1357-65.
39. Dawson-Hughes B, Harris SS. Calcium intake influences the association of protein intake with rates of bone loss in elderly men and women. Am J Clin Nutr 2002;75:773-9.
40. Smith SM, Wastney ME, Morukov BV, et al. Calcium metabolism before, during, and after a 3-mo spaceflight: kinetic and biochemical changes. Am J Physiol 1999;277:R1-10.

41. Smith SM, Wastney ME, O'Brien KO, et al. Bone markers, calcium metabolism, and calcium kinetics during extended-duration space flight on the Mir space station. J Bone Miner Res 2005;20:208-18.
42. Hayes J. The first decade of ISS exercise: lessons learned on Expeditions 1-25. Aerosp Med Hum Perform 2015;86:1-6.
43. Loerch LH. Exercise countermeasures on ISS: summary and future directions. Aerosp Med Hum Perform 2015;86:92-4.
44. Shackelford LC, LeBlanc AD, Driscoll TB, et al. Resistance exercise as a countermeasure to disuse-induced bone loss. J Appl Physiol 2004;97:119-129.
45. Remer T, Shi L, Alexy U. Potential renal acid load may more strongly affect bone size and mass than volumetric bone mineral density. Bone 2011;48:414-5.
46. Alexy U, Remer T, Manz F, Neu CM, Schoenau E. Long-term protein intake and dietary potential renal acid load are associated with bone modeling and remodeling at the proximal radius in healthy children. Am J Clin Nutr 2005;82:1107-14.
47. Baecker N, Tomic A, Mika C, et al. Bone resorption is induced on the second day of bed rest: results of a controlled crossover trial. J Appl Physiol 2003;95:977-82.
48. Buehlmeier J, Frings-Meuthen P, Remer T, et al. Alkaline salts to counteract bone resorption and protein wasting induced by high salt intake: results of a randomized controlled trial. J Clin Endocrinol Metab 2012;97:4789-97.
49. Frings-Meuthen P, Baecker N, Heer M. Low-grade metabolic acidosis may be the cause of sodium chloride-induced exaggerated bone resorption. J Bone Miner Res 2008;23:517-24.
50. Zwart SR, Smith SM. The impact of space flight on the human skeletal system and potential nutritional countermeasures. Intl SportMed J 2005;6:199-214.
51. Smith SM, Abrams SA, Davis-Street JE, et al. Fifty years of human space travel: implications for bone and calcium research. Annu Rev Nutr 2014;34:377-400.

Table 1. In-flight metabolism-related and dietary intake data for International Space Station crewmembers during 4-d controlled-intake and monitored-intake sessions[1]

Characteristic or outcome variable	**Pre (L-180)**		**Pre (L-45)**		**FD15**		**FD30**	**FD60**		**FD120**		**FD180[2]**	
	High (n=8)	Low (n=9)	High (n=8)	Low (n=8)	High (n = 8)	Low (n= 9)	Monitored (n = 17)	High (n=8)	Low (n=9)	High (n = 9)	Low (n = 8)	High (n=7)	Low (n = 7)
Body Mass (kg)	79±12	81±16	82±14	79±12	72±9[a]	86±14	79±13	79±14	80±13	88±9[a]	70±11	81±16	84±9
Female, n (%)	2 (25)	2 (22)	1 (13)	2 (25)	3 (38)	1 (11)	4 (24)	1 (13)	3 (33)	1 (11)	3 (38)	2 (29)	1 (14)
Cumulative 3-Week Exercise (1000 lb lifted)	-	-	-	-	327±147[b]	210±137[b]	528±302	703±412[b]	618±273	840±445[b]	501±174	644±295[b]	966±557[b]
Average CO_2 (mmHg)	-	-	-	-	3.38±0.44	3.08±0.38	3.13±0.43	3.25±0.41	2.94±0.54	2.69±0.89	2.61±0.58[b]	2.89±0.47	2.56±0.70
Energy (kcal/d)	2622±454[b]	2856±631[b]	2943±682[b]	2701±457[b]	2661±507[b]	2938±750[b]	2199±648	2829±691[b]	2675±594[b]	2992±698[b]	2435±378[b]	2399±471[a,b]	3303±651[b]
Energy/Body Mass (kcal/d/kg)	34±7	36±6	36±7	35±7	37±5	35±8	28±7	36±7	34±8	34±9	35±6	31±8	39±6
Total Protein (g)	116±11	115±26	128±23	111±12	109±16	124±25	98±35	126±25	107±17	130±20	100±14	110±15	131±25

Total Protein/Body Mass (kcal/d/kg)	1.49±0.21	1.45±0.25	1.58±0.25	1.43±0.22	1.51±0.16	1.47±0.28	1.23±0.39	1.61±0.28	1.35±0.18	1.48±0.24	1.45±0.23	1.38±0.22	1.56±0.24
Animal Protein (g)	84±8	56±15	95±19	54±8	81±12	61±14	67±29	94±19	52±9	94±17	48±6	81±12	63±13
Diet Potassium (mg)	2895±300	4723±810	3249±549	4612±535	2835±374	5094±785	3499±1036	3134±642	4469±523	3217±568	4179±394	2810±529	5046±933
APro:K	29±1	12±2	29±2	12±1	29±1	12±2	18±5	30±3	12±2	29±2	12±2	29±3	13±2
NEAP	86±11^{a,b}	44±11^{b}	91±14^{a,b}	42±3^{b}	84±10^{a,b}	46±10^{b}	57±14	94±14^{a,b}	46±11	96±13^{a,b}	44±9	88±10^{a,b}	54±9^{b}
Diet Sulfur (mEq/d)	64±7 ^{b}	55±12	71±12^{a,b}	54±6	61±10^{b}	59±12	51±19	70±13^{a,b}	52±8	72±11^{a,b}	48±7^{b}	61±8^{b}	63±11
Diet Calcium (mg)	1357±192	1369±178	1516±289	1427±203	1172±167	1315±242	1118±397	1252±169	1162±217	1218±240	1135±176	1117±177	1240±198
Diet Sodium (mg)	4644±1289	4203±642	4525±586	4358±1224	4375±921	4479±805	3726±1145	4631±591	4065±909	4251±995	4399±384	4506±575	3991±943
Diet Fiber (g)	22±2^{a,b}	42±7^{b}	25±4^{a}	42±6^{b}	21±3^{a}	45±8^{b}	28±7	24±5^{a,b}	40±6^{b}	25±4^{a,b}	38±5^{b}	21±3^{a}	48±8^{b}

[1] See Statistical Analyses section for full details. Briefly: measures in boldface were included in linear mixed-effects models comparing controlled preflight and in-flight sessions to the FD30 monitored session. A separate model using only the in-flight data controlled for exercise and CO_2, as these were not available before flight. All data are mean ± SD. APro:K, animal protein:potassium ratio; FD, flight day; High, high APro:K diet; Low, low APro:K diet; NEAP, net endogenous acid production; Pre (L-X), X days

before launch. [2]Not all astronauts were measured at FD180 because of flight duration, as described in Methods. [a]Significantly different from Low for the same FD, [b]significantly different from the monitored session.

Table 2. Bone markers in International Space Station crewmembers before and during flight, by 4-d controlled-intake dietary session[1]

Outcome variable		**Pre (L-180)**		**Pre (L-45)**		**Pre (L-10)**	**FD15**		**FD30**	**FD60**		**FD120**
Diet		High	Low	High	Low	Uncontrolled	High	Low	Monitored	High	Low	High
Urine	nmol/d	408±207	458±135	376±169	407±166	433±198	766±301	827±251	851±379	803±343	850±243	832±231
NTX	Δ	-43.7	40.7	-55.5	-45.1	-	340.9	386.4	417.4	432.7	360.1	314.4
		±220.4	±95.8	±70.8	±171.8		±161.1	±197.2	±314.0	±233.2	±231.6	±179.3
	% Δ	1±32	13±24	-14±18	0±27	-	85±35	111±76	108±74	130±70	91±57	71±45
Serum	U/L	22±7	26±7	25±7	25±7	28±6	24±6	29±5	28±5	39±5	31±5	38±10
BSAP	Δ	-4.0±5.1	-2.8±6.4	-3.9 ±6.4	-1.9±4.4	-	-0.8±1.7	-0.6±5.2	0.6±4.4	8.2±7.0	6.4±3.7	10.0 ±8.7
	% Δ	-15±19	-9±23	-13±22	-8±17	-	-3±7	-1±15	3±14	29±27	28±16	37±30
Urine	mmol/d	5±2	3±2	5±2	4±1	5±3	8±4	7±2	9±3	9±2	6±3	8±2
calcium	Δ	0.0±2.8	-2.1±2.5	-1.2 ±2.2	-1.2±2.0	-	3.7±2.5	1.9±3.4	3.5±2.8	3.4±3.3	0.8±2.4	1.3±2.6
	% Δ	24±83	-32±25	-12±24	-13±52	-	83±40	77±100	97±88	99±112	29±50	39±60

[1] Data are expressed as actual amount or concentration, as change (Δ) from the Pre (L-10) session when crewmembers were not on a controlled diet, and as percentage change (% Δ) from the Pre (L-10) session. Data are mean ± SD. APro:K, animal protein:potassium

ratio; BSAP, bone-specific alkaline phosphatase; FD, flight day; High, high APro:K diet; Low, low APro:K diet; NTX, N-telopeptide; Pre (L-X), X days before launch.

Table 3. Results of 2 linear mixed-effects models investigating the relationship of changes in urinary NTX excretion before and during spaceflight to HPK and LPK diets (preflight and in-flight model), or the relationship of changes in urinary NTX (relative to the monitored diet) only during flight to HPK and LPK diets (in-flight model).[1]

	Pre- and In-flight Model				**In-flight Model**				**In-flight Model without Outlier**			
Measure	**Estimate**	**Std. Error**	**t**	**p-value**	**Estimate**	**Std. Error**	**t**	**p-value**	**Estimate**	**Std. Error**	**t**	**p-value**
Intercept	-1231	348	-3.5	0.00	-1106	397	-2.8	0.01	-641	374	-1.7	0.09
HPK Diet	-147	75	-2.0	0.06	-218	80	-2.7	0.01	-120	78	-1.5	0.13
LPK Diet	-61	69	-0.9	0.39	-24	71	-0.3	0.74	32	67	0.5	0.64
Body Weight (kg)	0.2	2.5	0.1	0.93	3	3	1.2	0.26	1	3	0.5	0.61
Female	80	84	1.0	0.35	56	95	0.6	0.56	-18	86	-0.2	0.83
Age	18	6	3.1	<0.01	17	7	2.5	0.02	14	6	2.4	0.02
Energy (kcal/d)	0.00	0.00	0.3	0.80	0.04	0.07	0.6	0.56	-0.01	0.06	-0.1	0.89
NEAP	-2	2	-1.2	0.25	-1	2	-0.3	0.74	-1	2	-0.4	0.66
Dietary Sulfur (mEq/d)	8	3	2.3	0.03	6	3	1.8	0.08	5	3	1.5	0.15
Dietary Sodium (mg)	0.02	0.03	0.9	0.38	0.04	0.03	1.3	0.19	0.03	0.02	1.2	0.25
Dietary Fiber (g)	-2	5	-0.5	0.65	-5	4	-1.0	0.31	-3	4	-0.8	0.42
Flight Day	-0.2	0.2	-1.2	0.22	0.04	0.39	0.1	0.92	-0.26	0.38	-0.7	0.50
In-flight Status	453	67	6.7	<0.0001	-	-	-	-	-	-	-	-
Exercise	-	-	-	-	0.00	0.00	-0.7	0.47	0.00	0.00	0.1	0.94
Average CO_2	-	-	-	-	43	29	1.5	0.15	27	27	1.0	0.32
Random Effects												
$\sigma_{astronaut}$	86				100				87			
σ	134				115				105			

[1] Changes and percentage changes in urinary NTX excretion (Table 2) were between each 4-d controlled-diet session and the Pre (L-10) session when crewmembers were not on a controlled diet. The preflight and in-flight model controlled for body weight; sex; age; energy; NEAP; dietary sulfur, sodium, and fiber; flight day; and in-flight status. The in-flight model controlled for CO_2 and cumulative resistive exercise for 3 wk before data collection. Note: the data include all subjects, but 1 subject (outlier) was removed from these analyses because of an extremely high response during the FD30 session relative to the preflight baseline (as discussed in

Results, and shown in Supplemental Figure 1). APro:K, animal protein:potassium ratio; HPK, high APro:K; LPK, low APro:K; NEAP, net endogenous acid production; NTX, N-telopeptide.

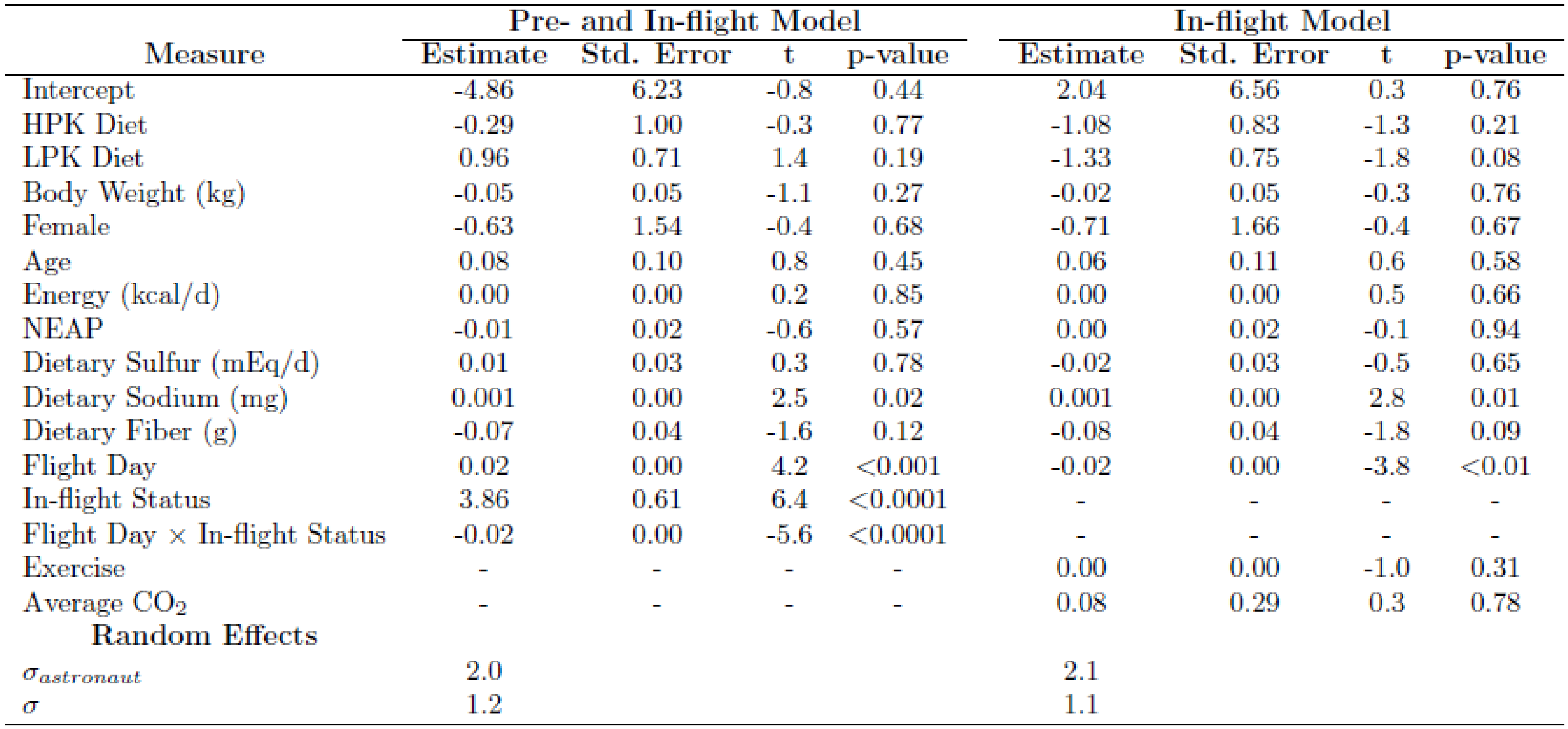
Table 4. Results of 2 linear mixed-effects models investigating the relationship of changes in serum BSAP before and during spaceflight to HPK and LPK diets (preflight and in-flight model), or the relationship of changes in BSAP (relative to the monitored diet) only during flight to HPK and LPK diets (in-flight model).[1]

	Pre- and In-flight Model				In-flight Model			
Measure	Estimate	Std. Error	t	p-value	Estimate	Std. Error	t	p-value
Intercept	-3.7	12.9	-0.3	0.77	-9.0	12.3	-0.7	0.47
HPK Diet	-2.7	3.8	-0.7	0.48	4.1	2.9	1.4	0.17
LPK Diet	-5.0	2.6	-1.9	0.06	1.9	2.6	0.7	0.48
Body Weight (kg)	-0.1	0.1	-1.1	0.28	-0.1	0.1	-0.9	0.40
Female	-3.9	2.9	-1.4	0.17	-2.5	2.9	-0.9	0.38
Age	0.3	0.2	1.5	0.15	0.1	0.2	0.7	0.49
Energy (kcal/d)	0.0	0.0	-0.8	0.41	0.0	0.0	-1.1	0.30
NEAP	-0.1	0.1	-1.8	0.08	0.0	0.1	-0.5	0.61
Dietary Sulfur (mEq/d)	0.1	0.1	0.8	0.45	0.0	0.1	-0.2	0.82
Dietary Sodium (mg)	0.0	0.0	-0.2	0.83	0.0	0.0	1.3	0.19
Dietary Fiber (g)	-0.1	0.2	-0.5	0.61	0.0	0.2	0.1	0.94
Flight Day	-0.1	0.0	-6.3	<0.0001	0.1	0.0	7.1	<0.001
In-flight Status	5.4	2.4	2.3	0.02	-	-	-	-
Flight Day × In-flight Status	0.1	0.0	8.0	<0.0001	-	-	-	-
Exercise	-	-	-	-	0.0	0.0	0.6	0.54
Average CO_2	-	-	-	-	3.0	1.1	2.7	0.01
Random Effects								
$\sigma_{astronaut}$	2.9				2.6			
σ	4.6				4.3			

[1] Changes and percentage changes in BSAP concentration (Table 2) were between each 4-d controlled-diet session and the Pre (L-10) session when crewmembers were not on a controlled diet. The preflight and in-flight model controlled for body weight; sex; age;

energy; NEAP; dietary sulfur, sodium, and fiber; flight day, and in-flight status. The in-flight model controlled for CO_2 and cumulative resistive exercise for 3 wk before data collection. APro:K, animal protein:potassium ratio; BSAP, bone-specific alkaline phosphatase; HPK, high APro:K; LPK, low APro:K; NEAP, net endogenous acid production.

Table 5. Results of 2 linear mixed-effects models investigating the relationship of changes in urinary calcium before and during spaceflight to HPK and LPK diets (preflight and in-flight model), or the relationship of changes in urinary calcium (relative to the monitored diet) only during flight to HPK and LPK diets (in-flight model).[1]



	Pre- and In-flight Model				In-flight Model			
Measure	Estimate	Std. Error	t	p-value	Estimate	Std. Error	t	p-value
Intercept	-4.86	6.23	-0.8	0.44	2.04	6.56	0.3	0.76
HPK Diet	-0.29	1.00	-0.3	0.77	-1.08	0.83	-1.3	0.21
LPK Diet	0.96	0.71	1.4	0.19	-1.33	0.75	-1.8	0.08
Body Weight (kg)	-0.05	0.05	-1.1	0.27	-0.02	0.05	-0.3	0.76
Female	-0.63	1.54	-0.4	0.68	-0.71	1.66	-0.4	0.67
Age	0.08	0.10	0.8	0.45	0.06	0.11	0.6	0.58
Energy (kcal/d)	0.00	0.00	0.2	0.85	0.00	0.00	0.5	0.66
NEAP	-0.01	0.02	-0.6	0.57	0.00	0.02	-0.1	0.94
Dietary Sulfur (mEq/d)	0.01	0.03	0.3	0.78	-0.02	0.03	-0.5	0.65
Dietary Sodium (mg)	0.001	0.00	2.5	0.02	0.001	0.00	2.8	0.01
Dietary Fiber (g)	-0.07	0.04	-1.6	0.12	-0.08	0.04	-1.8	0.09
Flight Day	0.02	0.00	4.2	<0.001	-0.02	0.00	-3.8	<0.01
In-flight Status	3.86	0.61	6.4	<0.0001	-	-	-	-
Flight Day × In-flight Status	-0.02	0.00	-5.6	<0.0001	-	-	-	-
Exercise	-	-	-	-	0.00	0.00	-1.0	0.31
Average CO_2	-	-	-	-	0.08	0.29	0.3	0.78
Random Effects								
$\sigma_{astronaut}$	2.0				2.1			
σ	1.2				1.1			

[1] Changes and percentage changes in urinary calcium concentration (Table 2) were between each 4-d controlled-diet session and the Pre (L-10) session when crewmembers were not on a controlled diet. The preflight and in-flight model controlled for body weight; sex; age; energy; NEAP; dietary sulfur, sodium, and fiber; flight day, and in-flight status. The in-flight model controlled for average CO_2 and cumulative resistive exercise for 3 wk before data collection. APro:K, animal protein:potassium ratio; BSAP, bone-specific alkaline phosphatase; HPK, high APro:K; LPK, low APro:K; NEAP, net endogenous acid production.

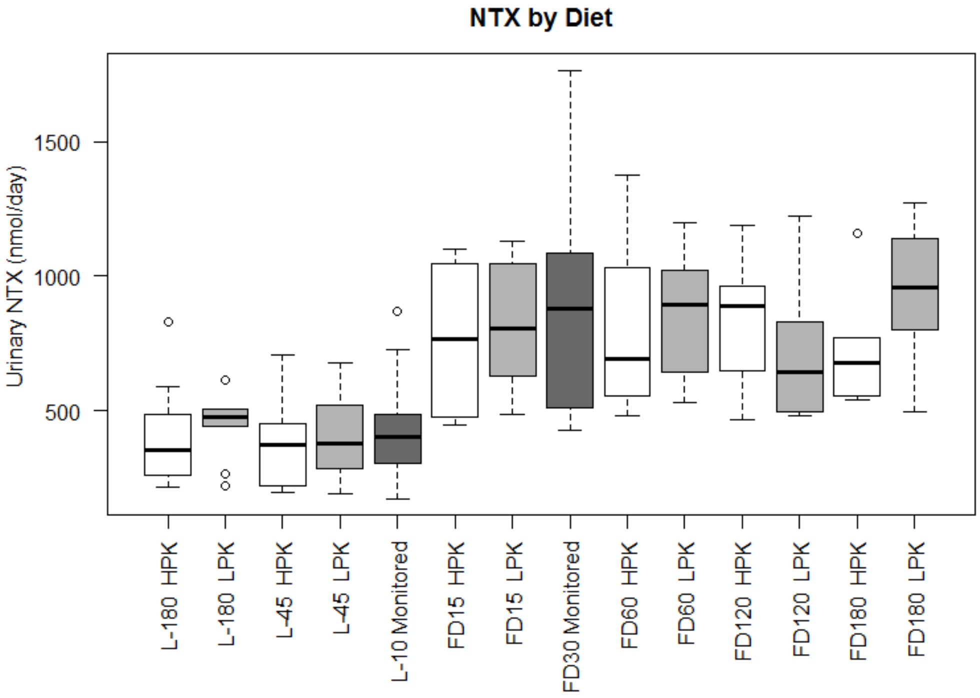
Legends for figures

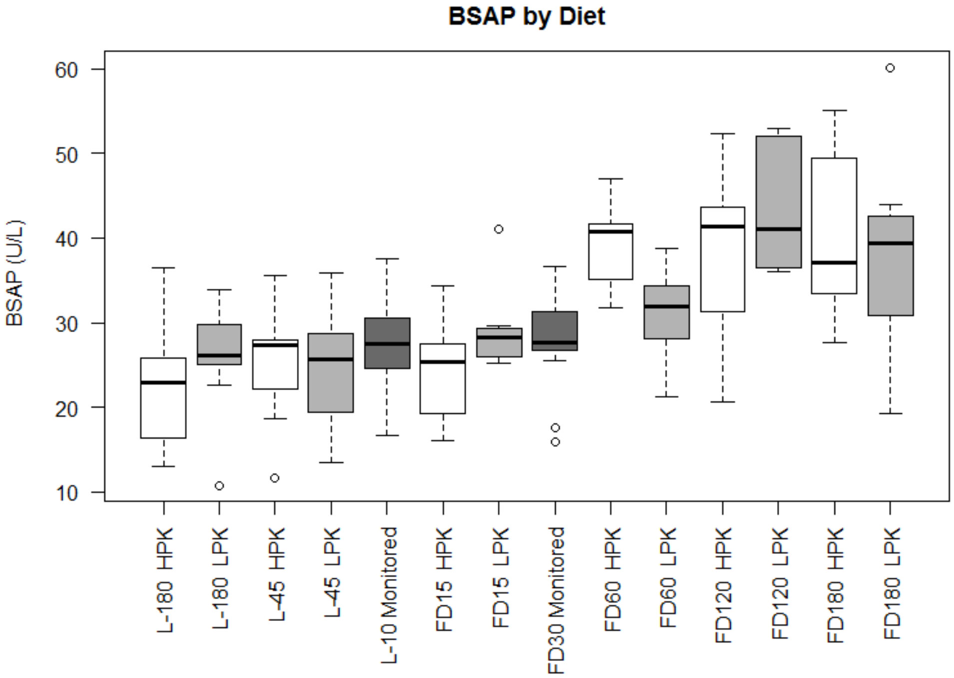
FIGURE 1. Box plots of urinary NTX and calcium, and serum BSAP of crewmembers consuming a 4-d diet with controlled HPK or LPK intake, or an intake that was not controlled but monitored, on the indicated days before and during spaceflight. Black lines indicate median values for each group. Boxes represent interquartile range (IQR). Small circles indicate observations outside 1.5*IQR. Box shading: no shading, HPK diet; light shading, LPK diet; heavy shading, monitored diet. APro:K, animal protein:potassium ratio; BSAP, bone-specific alkaline phosphatase; FD, flight day; HPK, high APro:K diet; LPK, low APro:K diet; L-X, the Xth day before launch; NTX, N-telopeptide.

FIGURE 2. Dietary NEAP estimated from crewmembers' diet for 4 d collected around FD30, used as a proxy for their typical intake throughout their mission, was related to the change in lumbar spine BMC after flight ($P < 0.01$). Each point represents a single crewmember. The association remained significant after adjusting for age and sex ($P < 0.05$). BMC, bone mineral content; L, lumbar; NEAP, net endogenous acid production.

FIGURE 3. Relationship of NTX to dietary APro:K before (Pre) and during bed rest in sedentary (Control, $n = 6$) and exercising subjects ($n = 5$).

Figure 1. Box plots of urinary NTX and calcium, and serum BSAP of crewmembers consuming a 4-d diet with controlled HPK or LPK intake, or an intake that was not controlled but monitored, on the indicated days before and during spaceflight. Black lines indicate median values for each group. Boxes represent interquartile range (IQR). Small circles indicate observations outside 1.5*IQR. Box shading: no shading, HPK diet; light shading, LPK diet; heavy shading, monitored diet. APro:K, animal protein:potassium ratio; BSAP, bone-specific alkaline phosphatase; FD, flight day; HPK, high APro:K diet; LPK, low APro:K diet; L-X, the Xth day before launch; NTX, N-telopeptide.




BSAP by Diet
BSAP (U/L)
60
50
40
30
20
10
L-180 HPK
L-180 LPK
L-45 HPK
L-45 LPK
L-10 Monitored
FD15 HPK
FD15 LPK
FD30 Monitored
FD60 HPK
FD60 LPK
FD120 HPK
FD120 LPK
FD180 HPK
FD180 LPK

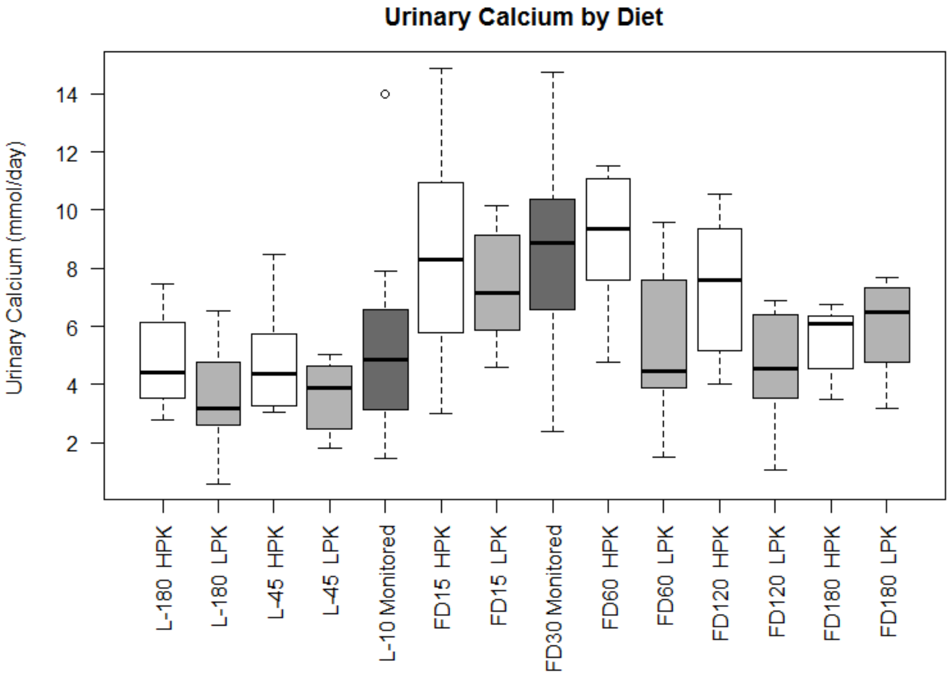

Urinary Calcium by Diet
Urinary Calcium (mmol/day)
14
12
10
8
6
4
2
L-180 HPK
L-180 LPK
L-45 HPK
L-45 LPK
L-10 Monitored
FD15 HPK
FD15 LPK
FD30 Monitored
FD60 HPK
FD60 LPK
FD120 HPK
FD120 LPK
FD180 HPK
FD180 LPK

Figure 2. Dietary NEAP estimated from crewmembers' diet for 4 d collected around FD30, used as a proxy for their typical intake throughout their mission, is related to the change in lumbar spine BMC after flight (*P* < 0.01). Each point represents a single crewmember. The association remained significant after adjusting for age and sex (*P* < 0.05). BMC, bone mineral content; L, lumbar; NEAP, net endogenous acid production.

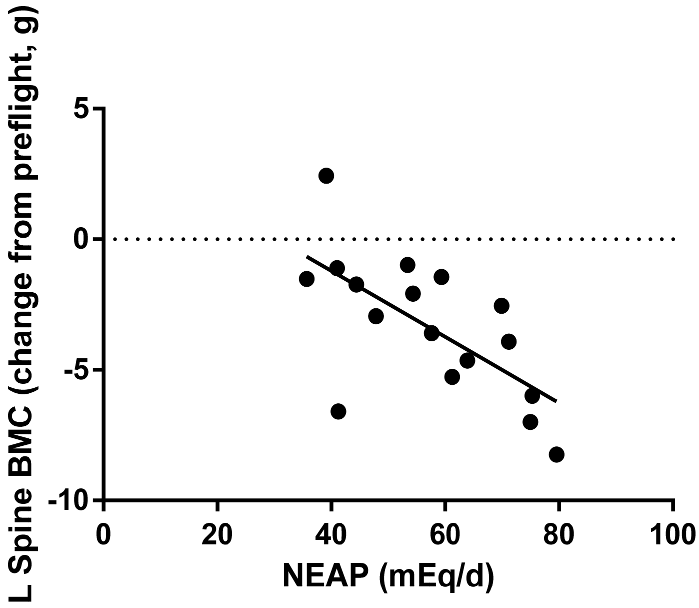
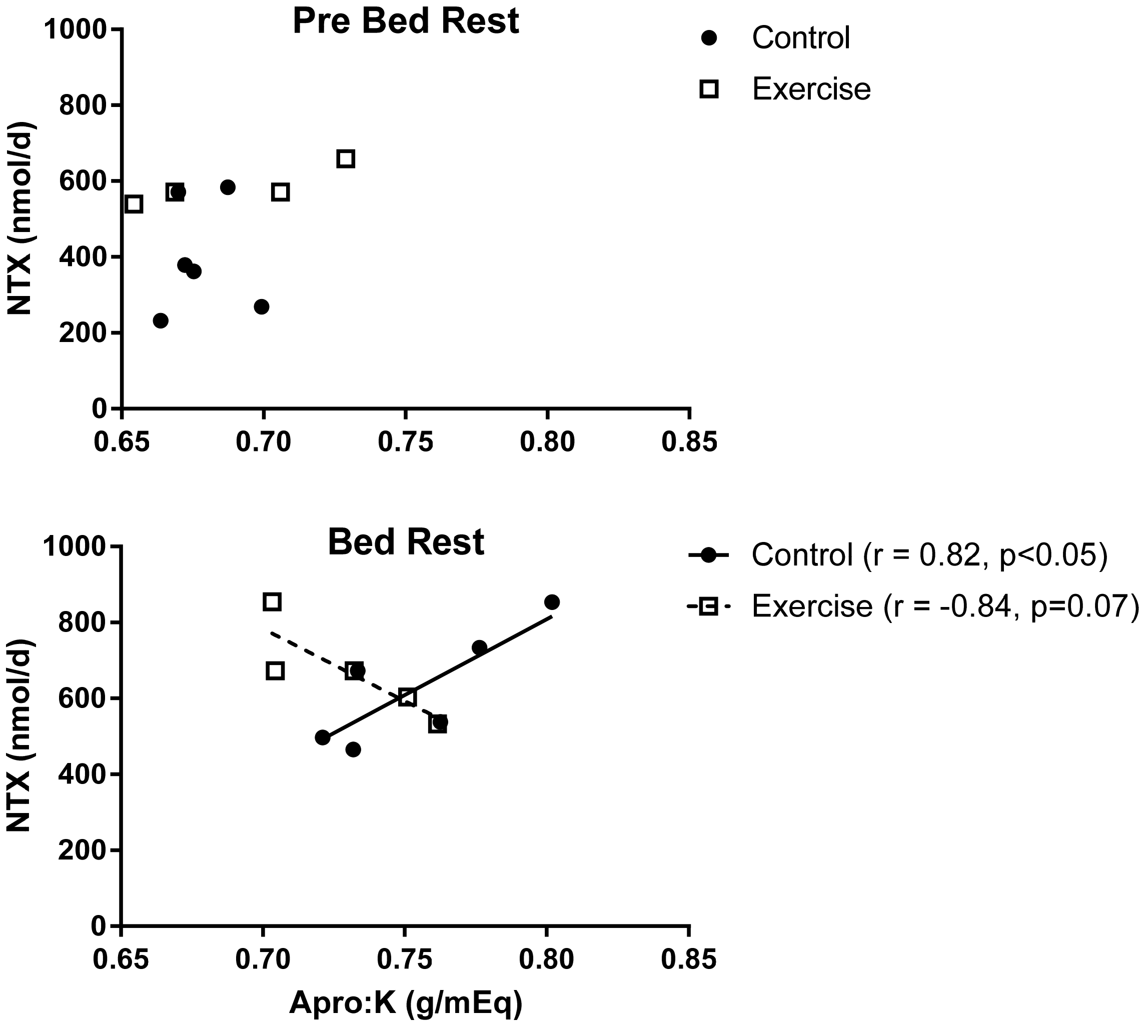


Figure 3. Dietary APro:K before and during bed rest in sedentary (n=6) and exercising subjects (n=5).



Supplemental Table 1. Dietary intake before and during spaceflight[1]

	Pre (L-180)		Pre (L-45)		FD15		FD30	FD60		FD120		FD180*	
Dietary intake	High (n=8)	Low (n=9)	High (n=8)	Low (n=8)	High (n = 8)	Low (n= 9)	Monitored (n = 17)	High (n=8)	Low (n=9)	High (n = 9)	Low (n = 8)	High (n=7)	Low (n = 7)
Total Protein (g)	116±11	115±26	128±23	111±12	109±16	124±25	98±35	126±25	107±17	130±20	100±14	110±15	131±25
Animal Protein (g)	84±8	56±15	95±19	54±8	81±12	61±14	67±29	94±19	52±9	94±17	48±6	81±12	63±13
Vegetable Protein (g)	31±5	59±14	32±7	56±9	27±6	63±16	31±9	32±8	54±11	34±7	51±12	28±7	68±17
Diet Calcium (mg)	1357±192	1369±178	1516±289	1427±203	1172±167	1315±242	1118±397	1252±169	1162±217	1218±240	1135±176	1117±177	1240±198
Diet Sodium (mg)	4644±1289	4203±642	4525±586	4358±1224	4375±921	4479±805	3726±1145	4631±591	4065±909	4251±995	4399±384	4506±575	3991±943
Cholesterol (mg)	412±126	212±64	434±108	231±64	394±137	216±56	302±150	448±104	234±82	403±107	219±71	439±99	220±57
Total Saturated Fatty Acids (SFA) (g)	31±8	21±5	33±7	23±8	31±7	23±9	25±10	31±8	24±9	34±8	21±6	28±9	26±6
Total Monounsaturated Fatty Acids (MUFA)(g)	35±10	51±19	36±9	50±14	33±10	59±23	29±9	37±12	46±12	37±10	42±12	31±9	63±21
Total Polyunsaturated Fatty Acids (PUFA) (g)	18±5	27±7	19±3	25±7	18±4	28±11	16±5	19±6	25±7	19±4	23±8	16±5	33±11

Total Dietary Fiber (g)	22±2	42±7	25±4	42±6	21±3	45±8	28±7	24±5	40±6	25±4	38±5	21±3	48±8
Total Fat (g)	90±21	106±31	94±17	104±23	89±20	116±41	76±26	92±25	101±22	97±19	91±25	80±20	128±37
Total Carbohydrate (g)	341±82	362±82	397±123	334±76	357±86	353±93	288±84	374±109	336±100	403±131	306±51	310±88	409±79
Total Vitamin A Activity (International Units) (IU)	6320±2026	12647±6314	8246±4496	14364±3698	9210±3690	15896±5946	9835±4179	6824±2111	12196±3698	6909±4534	12916±4503	5615±2683	12543±5942
Vitamin D (mcg)	4±1	3±1	5±1	3±1	6±4	10±14	14±13	8±6	3±2	6±5	5±7	8±7	5±3
Total Alpha-Tocopherol Equivalents (mg)	15±4	30±9	18±5	25±9	14±4	36±19	22±10	18±6	27±9	21±9	24±9	14±4	38±10
Vitamin K (mcg)	81±32	142±42	99±36	166±29	82±35	174±32	126±56	85±24	142±33	78±28	153±31	99±35	141±27
Vitamin C (mg)	78±38	159±70	102±94	162±50	61±18	183±58	134±52	119±86	146±45	93±91	131±57	67±27	184±54
Thiamin (mg)	2.13±0.45	2.31±0.60	2.31±0.57	2.24±0.52	2.03±0.54	2.73±0.77	1.87±0.60	2.27±0.59	2.09±0.41	2.56±0.58	2.00±0.23	2.01±0.27	2.70±0.67
Riboflavin (mg)	2.42±0.42	2.23±0.32	2.56±0.48	2.19±0.33	2.22±0.45	2.52±0.61	2.16±0.69	2.62±0.57	2.14±0.33	2.66±0.65	2.05±0.32	2.28±0.25	2.58±0.37
Niacin (mg)	27.08±4.20	28.44±8.45	29.77±9.25	28.43±3.95	25.39±7.17	34.28±8.02	25.64±9.30	31.53±8.20	25.74±4.43	32.73±7.04	25.26±5.36	23.53±5.21	34.81±7.45
Pantothenic acid (mg)	5.45±0.68	5.41±0.74	5.97±0.50	5.62±1.08	5.17±0.61	7.34±3.14	6.63±2.93	5.73±0.83	5.17±0.87	6.41±1.59	5.21±0.51	5.39±0.68	6.03±1.17
Vitamin B-6 (mg)	1.97±0.31	2.25±0.59	2.30±0.69	2.24±0.30	1.82±0.47	2.90±1.02	2.35±0.95	2.40±0.58	2.09±0.36	2.47±0.67	1.96±0.21	1.76±0.28	2.75±0.48
Folate (mcg)	354±87	592±115	391±79	549±72	340±91	672±188	441±165	392±88	523±71	399±150	526±78	333±37	662±143

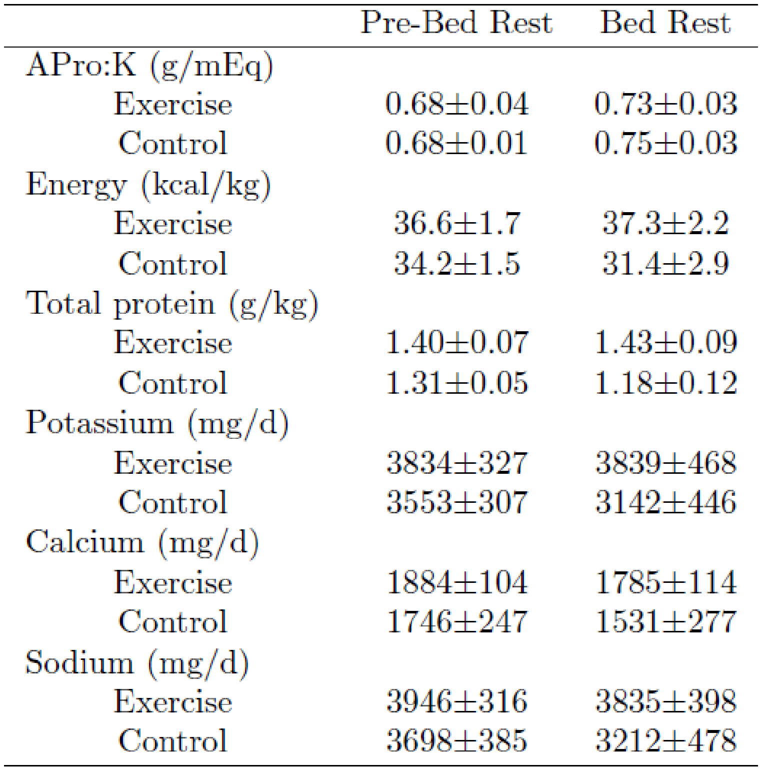
Vitamin B-12 (mcg)	5.38±0.91	4.69±1.59	6.11±1.07	4.07±1.11	5.53±0.96	8.18±8.04	7.15±5.92	6.28±1.09	3.98±0.92	7.06±4.31	4.06±1.07	4.87±0.97	6.62±2.36
Choline (mg)	437±65	398±75	492±79	384±63	412±71	419±93	366±136	486±76	372±67	479±81	354±50	448±68	436±81
Phosphorus (mg)	1955±290	2017±353	2169±291	2004±244	1876±279	2136±372	1639±506	2099±386	1940±302	2096±357	1836±277	1877±239	2222±375
Magnesium (mg)	419±54	674±145	427±64	662±91	342±48	701±134	392±111	397±73	589±99	415±63	544±92	353±66	716±149
Iron (mg)	17±2	22±4	20±5	23±5	15±3	24±4	17±5	20±4	21±4	19±4	21±3	16±3	26±6
Zinc (mg)	15±3	15±3	16±3	14±2	13±2	18±5	14±4	16±4	13±2	17±3	13±1	15±2	17±3
Copper (mg)	2.04±0.45	2.98±0.49	2.19±0.41	3.14±0.62	1.76±0.28	3.52±0.62	2.05±0.50	2.13±0.45	2.77±0.47	2.22±0.46	2.71±0.36	1.82±0.19	3.37±0.58
Selenium (mcg)	176±31	134±32	196±41	135±28	181±34	142±32	137±49	195±46	127±29	198±39	121±22	163±18	155±32
Sodium (mg)	4644±1289	4203±642	4525±586	4358±1224	4375±921	4479±805	3726±1145	4631±591	4065±909	4251±995	4399±384	4506±575	3991±943
Potassium (mg)	2895±300	4723±810	3249±549	4612±535	2835±374	5094±785	3499±1036	3134±642	4469±523	3217±568	4179±394	2810±529	5046±933
Manganese (mg)	4.63±0.48	8.15±2.49	5.00±1.34	7.99±1.31	4.16±0.91	9.04±2.41	5.17±1.67	5.05±1.30	7.33±1.77	5.26±1.10	6.74±1.14	4.45±0.64	9.68±1.98
PUFA 18:2 (linoleic acid) (g)	16±5	25±7	17±3	23±7	16±4	26±10	14±5	17±5	23±7	17±4	21±8	14±4	30±10
PUFA 18:3 (linolenic acid) (g)	1.41±0.44	1.70±0.38	1.45±0.19	1.61±0.50	1.57±0.34	1.62±0.63	1.23±0.43	1.44±0.39	1.62±0.34	1.44±0.33	1.55±0.63	1.21±0.28	2.10±0.51

PUFA 20:5 (eicosapentaenoic acid [EPA]) (g)	0.09±0.09	0.07±0.04	0.06±0.05	0.11±0.10	0.10±0.06	0.09±0.10	0.08±0.07	0.06±0.05	0.07±0.05	0.06±0.06	0.09±0.06	0.07±0.05	0.06±0.05
PUFA 22:6 (docosahexaenoic acid [DHA]) (g)	0.13±0.09	0.11±0.03	0.12±0.04	0.15±0.15	0.16±0.06	0.14±0.13	0.14±0.14	0.12±0.08	0.09±0.05	0.11±0.06	0.14±0.11	0.09±0.05	0.12±0.06
Methionine (g)	2.76±0.29	2.26±0.54	3.09±0.60	2.22±0.27	2.65±0.43	2.42±0.51	2.21±0.88	3.04±0.61	2.10±0.34	3.11±0.53	1.97±0.27	2.63±0.35	2.57±0.47
Cystine (g)	1.61±0.20	1.51±0.32	1.78±0.27	1.44±0.15	1.53±0.26	1.58±0.34	1.31±0.45	1.75±0.33	1.41±0.23	1.80±0.26	1.32±0.22	1.56±0.20	1.70±0.32
Caffeine (mg)	84±68	102±64	83±61	99±90	67±52	114±79	120±126	67±40	127±90	131±88	89±78	174±171	72±54
Phytic Acid (mg)	949±244	2202±637	987±348	2038±519	764±208	2317±833	955±357	985±372	2025±509	1090±337	1799±610	883±325	2637±802
Oxalic Acid (mg)	226±54	538±136	231±61	559±105	201±57	623±102	255±104	237±69	521±146	246±61	483±115	216±59	602±137
Water (g)	4031±1209	4267±887	4467±1471	4659±1119	3159±757	2889±663	2654±675	2904±865	3377±727	3230±1045	3037±477	3114±424	3622±1015
Beta-Carotene (mcg)	2432±1054	6048±3289	3451±2773	6741±1877	4157±2322	7414±3017	4229±2045	2434±1075	5770±1992	2724±2735	6042±2369	2261±1303	5947±3086
Lutein + Zeaxanthin (mcg)	1414±488	3013±852	1621±569	3138±822	1549±568	3731±575	2325±1310	1519±321	2824±1032	1333±322	2795±398	1541±620	2888±962
Lycopene (mcg)	2151±2679	5357±1708	1402±1188	8148±2698	2451±2424	7415±2380	4300±2809	1761±2602	6733±3303	1125±1022	5726±1435	1556±1539	6121±2683
Omega-3 Fatty Acids (g)	1.67±0.60	1.89±0.38	1.66±0.22	1.90±0.60	1.88±0.42	1.96±0.74	1.45±0.64	1.67±0.52	1.79±0.33	1.65±0.41	1.81±0.63	1.39±0.34	2.21±0.61

Soy Isoflavones (daidzein+genistein+glycitein mg)	4.06±3.90	15.83±6.37	3.52±3.02	21.59±11.01	2.55±1.57	22.65±7.29	5.71±5.53	4.21±3.61	15.16±7.63	3.38±3.42	19.35±7.38	4.82±4.54	16.21±6.53
Flavonoids (Coumestrol, Biochanin A, Formononetin, mg)	0.00±0.00	0.57±0.63	0.31±0.56	0.59±0.63	0.16±0.43	0.45±0.53	0.48±0.72	0.16±0.43	0.39±0.57	0.14±0.40	1.06±0.58	0.01±0.01	0.35±0.48
Added Sugars (g)	143±55	132±43	172±68	100±38	155±50	113±47	105±46	158±61	114±53	175±77	101±35	129±63	145±37

[1] Data are mean ± SD. APro:K, animal protein:potassium ratio; FD, flight day; High, high APro:K diet; Low, low APro:K diet; Pre (L-X), X days before launch; PUFA, polyunsaturated fatty acids. *Not all astronauts were measured at FD180 because of flight duration

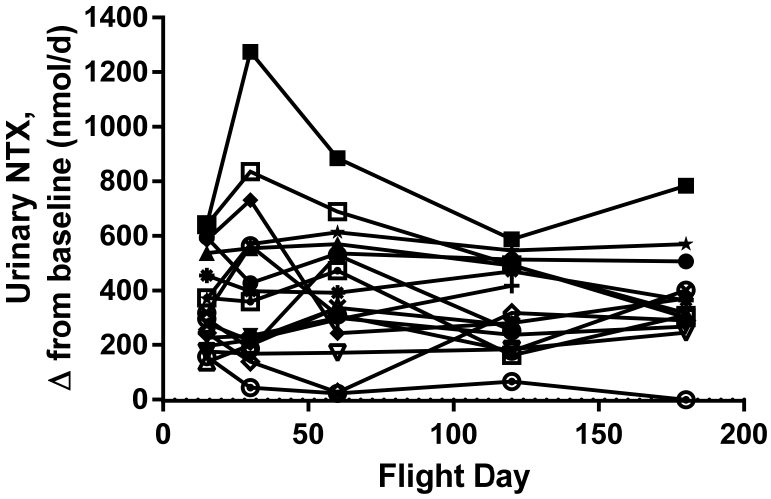
Supplemental Table 2. Dietary intake data before (Pre-Bed Rest) and after (Bed Rest) the 70-d bed rest study[1]



	Pre-Bed Rest	Bed Rest
APro:K (g/mEq)		
Exercise	0.68±0.04	0.73±0.03
Control	0.68±0.01	0.75±0.03
Energy (kcal/kg)		
Exercise	36.6±1.7	37.3±2.2
Control	34.2±1.5	31.4±2.9
Total protein (g/kg)		
Exercise	1.40±0.07	1.43±0.09
Control	1.31±0.05	1.18±0.12
Potassium (mg/d)		
Exercise	3834±327	3839±468
Control	3553±307	3142±446
Calcium (mg/d)		
Exercise	1884±104	1785±114
Control	1746±247	1531±277
Sodium (mg/d)		
Exercise	3946±316	3835±398
Control	3698±385	3212±478

[1] The data are 7-d averages ± SD before bed rest and 7-d averages ± SD during the last week of bed rest. APro:K, animal protein:potassium ratio.

Supplemental Figure 1. Change in urinary NTX of individual astronauts from the preflight baseline[1]



[1] The flight dates shown are approximate, for de-identification of the subjects. NTX, N-telopeptide.